Supporting Information

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SI Text

Preparation of PEG800 and NIR Fluorescence Imaging. NIR fluorophore-conjugated PEG800 was synthesized by conjugation of 20 nmol of *N*-hydroxysuccinimide ester of IRDye 800-CW (LI-COR, Lincoln, NE) to 10 nmol of 5-kDa amino monomethoxy PEG (Nektar, Huntsville, AL) in phosphate buffer, pH 7.8. After purification of the product (PEG800) using gel-filtration chromatography on a 6 kDa MWCO Econo-Pac P6 cartridge (Bio-Rad, Hercules, CA), it was lyophilized and resuspended at a final concentration of 10 μM in PBS, pH 7.4 before injection. *In vivo* NIR fluorescence imaging was performed as described in detail previously (1) after i.v. injection of 0.5 nmol of PEG800.

Primary Endothelial Cell Isolation. Endothelial cells were isolated from p85αflox/flox; p85$\beta^{-/-}$ adult mouse lungs. Four lungs were dissected and washed with cold HBSS with antibiotics. The tissue was minced into 2-mm pieces and transferred into tubes containing 15 ml of warm 0.1% collagenase type I (Worthington) in DMEM with antibiotics. Tissues were digested for 30 min at 37°C with agitation. Tissues were pipetted up and down to break up clumps and filtered through a 70 μm filter. Cells were pelleted at 100 $\times$ *g* for 5 min and resuspended in PBS/0.1% BSA/1 mM $CaCl_2$/0.5 mM $MgCl_2$/120 Kunitz DNAse1/ml. The cells were rocked for 15 min at room temperature to eliminate DNA from lysed cells. After pelleting, the cells were washed twice in wash buffer (PBS without Ca^{2+}/Mg^{2+}/0.1% BSA/2 mM EDTA) and resuspended in 1.5 ml of wash buffer with 2.5 μg of rat anti-mouse CD31 antibody (BD Biosciences). Cells were rocked gently for 20 min at 4°C and then washed with 2 ml of wash buffer. Cells were resuspended in 2 ml wash buffer and 25 μl Dynabeads M450 sheep anti-rat IgG (Invitrogen) was added. Cells and beads were rocked for 20 min at 4°C followed by magnetic separation of cell-bead complexes. Beads were washed four times in wash buffer and plated on 0.1% gelatin-coated plates. Cells were fed every 2 days with EBM-2 (Lonza)/ 0.05% Endothelial Cell Mitogen (Biomedical Technologies, Inc.)/ 20% inactivated FBS/ penicillin/streptomycin. After 2 weeks of growth, cells were immortalized by retroviral infection of a p53 short hairpin construct followed by a second round of magnetic bead separation as described above.

1. Nakayama A, del Monte F, Hajjar RJ, Frangioni JV (2002) Functional near-infrared fluorescence imaging for cardiac surgery and targeted gene therapy. *Mol Imaging* 1(4):365–377.

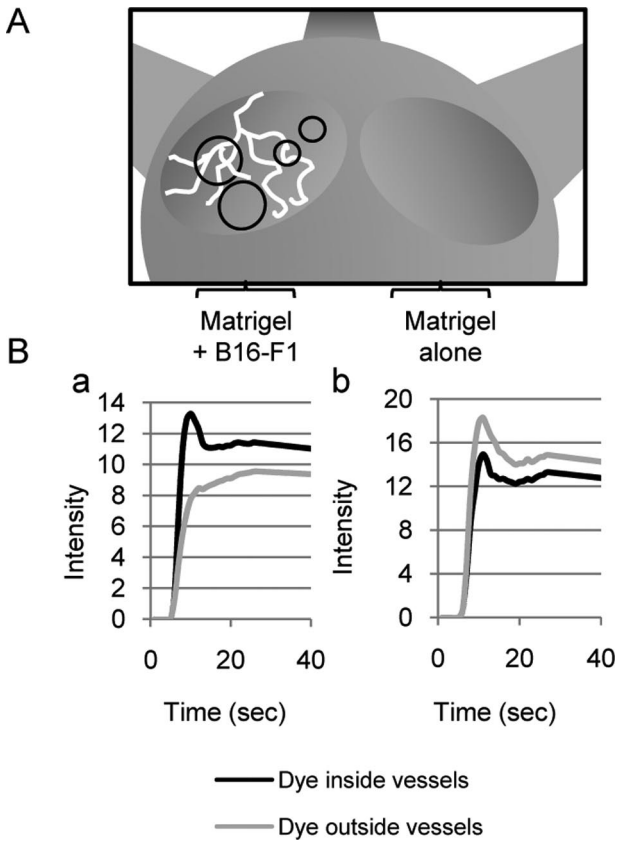

Fig. S1. Method of dye extravasation quantification. (*A*) NIR intensity was measured within demarcated regions of interest (ROI) representing intravascular and extravascular regions, as illustrated. (*B*) NIR intensity was quantified in ROIs over time. (*a*) More NIR dye is enclosed within vessels at all time points. (*b*) Rapid extravasation of dye to extravascular regions. Results were consistent when analyzing small or large ROIs. These intensity values were used to determine permeability ratios in Fig. 3 *B* and *C*.

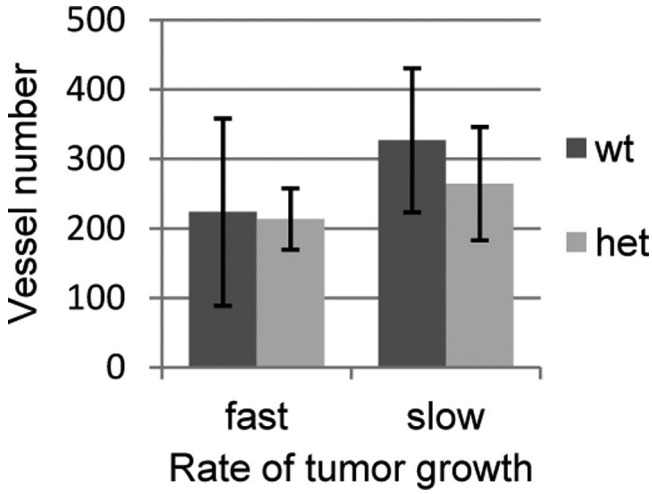

Fig. S2. Vessel density within tumors was measured by counting CD31-positive structures in tumor sections. There was no difference in vessel density between wild-type and heterozygous mice, regardless of the rate of tumor growth.